Melatonin and Dopamine as Biomarkers to Optimize Treatment in Phenylketonuria: Effects of Tryptophan and Tyrosine Supplementation

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Objective To determine whether additional supplementation of tryptophan (Trp) and tyrosine (Tyr) improve serotonin and dopamine metabolism in individuals with phenylketonuria treated with large neutral amino acid (LNAA) tablets.

Study design Ten adult individuals with phenylketonuria participated in a randomized, double-blind, placebo-controlled cross-over study consisting of three 3-week phases: washout, treatment with LNAA tablets plus supplementation with either Trp and Tyr tablets or placebo, and LNAA tablets plus the alternate supplementation. An overnight protocol to measure blood melatonin, a serotonin metabolite in the pinealocytes, and urine 6-sulfatoxymelatonin and dopamine in first-void urine specimens was conducted after each phase.

Results Serum melatonin and urine 6-sulfatoxymelatonin and dopamine levels were increased in the LNAA phase (LNAA plus placebo) compared with the washout phase. Serum melatonin and urine 6-sulfatoxymelatonin were not increased in the active phase (LNAA plus Trp + Tyr) compared with the LNAA phase, although plasma Trp:LNAA was increased compared with the LNAA phase. Among 7 subjects with a plasma Trp/LNAA >0.03, a negative correlation between urine 6-sulfatoxymelatonin and plasma phenylalanine levels was observed ($r = -0.072$). Urine dopamine levels and plasma Tyr:LNAA were increased in the active phase compared with the LNAA phase.

Conclusion Melatonin levels were not increased with the higher dose of Trp supplementation, but dopamine levels were increased with the higher dose of Tyr supplementation. Serotonin synthesis appears to be suppressed by high phenylalanine levels at the Trp hydroxylase level. *(J Pediatr 2014;165:184-89)*.

The dietary treatment of phenylketonuria (PKU) has been based primarily on restricting phenylalanine (Phe) intake to maintain blood Phe levels within the recommended range. Blood Phe levels of 2-6 mg/dL (120-360 μM) for children age <12 years and <15 mg/dL (<900 μM) for individuals age >12 years are widely recommended.[1] Neuropsychological studies of individuals with PKU who are diagnosed early and well controlled have shown a higher prevalence of characteristic deficits, such as decreased executive functioning and internalizing disorders.[2,3] Abnormal metabolism of neurotransmitters, particularly serotonin and dopamine, is frequently observed in individuals with PKU and is believed to be involved in these psychological and psychiatric symptoms. A direct correlation between blood Phe level and neurotransmitter levels in the central nervous system (CNS) has not been established. Because melatonin is synthesized from serotonin in the pinealocytes in the CNS, in our previous study[4] we measured this metabolite as a biomarker to reflect serotonin synthesis in the CNS.

The transport of tryptophan (Trp) from blood into the pinealocytes is achieved through the monocarboxylate transporter 10 or T-type amino acid transporter 1.[5] Both Trp and tyrosine (Tyr) are transported into the brain by a shared large neutral amino acid transporter 1 (LAT1) at the blood-brain barrier, and their uptake is inhibited by high levels of competing large neutral amino acids (LNAAs) such as Phe. Trp concentration is the most important single metabolic determinant in brain serotonin synthesis, because Trp hydroxylase (TPH; EC 1.14.16.4) is unsaturated at the physiological concentration of Trp in the brain.[6] In our previous study, we found low 6-sulfatoxymelatonin and dopamine levels in first-void urine in the study subjects with PKU compared with controls.[4] When diets were supplemented with LNAA tablets (PheBloc Applied Nutrition, Los Angeles, California), providing 30 mg/kg/day of Trp and 100 mg/kg/day of Tyr, these levels increased and Trp:LNAA and Tyr:LNAA improved, although they were significantly lower than those in controls.[4] Although these 2 urine biomarker levels were

AUC	Area under the curve
CNS	Central nervous system
LAT1	Large neutral amino acid transporter 1
LNAA	Large neutral amino acid
LNAA + TT	Large neutral amino acid with tryptophan/tyrosine supplementation
Phe	Phenylalanine
PKU	Phenylketonuria
TH	Tyrosine hydroxylase
TPH	Tryptophan hydroxylase
Trp	Tryptophan
Tyr	Tyrosine

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increased after increases in plasma Trp:LNAA and Tyr:LNAA after supplementation of LNAA tablets, they did not reach the control levels. In this study, additional dosages of Trp and Tyr were provided to individuals with PKU, along with LNAA tablets, to determine whether further increases in 6-sulfatoxymelatonin and dopamine levels, likely indicating improvement in the CNS serotonin and dopamine metabolism, could be seen.

Methods

Ten adult individuals (2 females and 8 males) with classical PKU were enrolled after providing informed consent. The study protocol was approved by the University of Southern California's Health Science Institutional Review Board. Study subjects were recruited from our outpatient population and ranged in age from 21 to 51 years (mean ± SD, 29.4 ± 9.4 years). The study was conducted in three 3-week phases. After an initial washout phase without use of any medical food products, the subjects were given LNAA tablets during the next two 3-week phases. They were randomly assigned in a double-blind manner to take tablets consisting of either additional Trp and Tyr or placebo during the next 3 weeks, and then cross over to the alternate supplementation for the final 3 weeks. They consumed a regular diet but avoided high-protein foods, and no dietary changes were made throughout the study. The number of LNAA tablets (PheBloc; Applied Nutrition, Cedar Knolls, New Jersey) taken daily, following the manufacturer's recommendation, was based on the subject's weight (body weight in kg × 0.5; maximum total daily intake, 45 tablets/day) and provided the following doses of LNAAs: Trp, 30.6 mg/kg/day; Tyr, 98.4 mg/kg/day; histidine, 15.6 mg/kg/day; isoleucine, 15.7 mg/kg/day; leucine, 15.4 mg/kg/day; methionine, 24.8 mg/kg/day; threonine, 16.4 mg/kg/day; valine, 16 mg/kg/day; and Phe, 0 mg/kg/day. Trp/Tyr tablets and placebo tablets were manufactured by Applied Nutrition for this study. During the active phase, subjects were further supplemented with Trp/Tyr tablets providing an additional 69.4 mg/kg/day of Trp and 101.6 mg/kg/day of Tyr, to receive a total of 100 mg/kg/day of Trp and 200 mg/kg/day of Tyr.

The study subjects stayed overnight at the Clinical Trials Unit at the University of Southern California's University Hospital at the end of each phase. All subjects received the same protein-controlled meal during the overnight evaluation. Serum melatonin was measured every 2 hours from 7:00 p.m. to 7:00 a.m., and first-void urine specimens were collected at 7:00 a.m. to measure dopamine and 6-sulfatoxymelatonin, to which 80%-90% of melatonin is metabolized and excreted into urine.[6] Specimens for plasma amino acid measurements were obtained before dinner at 7:00 p.m. Serum and urine specimens were processed and kept in a freezer (−20°C) until analysis. Serum melatonin and urine 6-sulfatoxymelatonin were measured as described elsewhere,[7] and plasma amino acids were measured by high-performance liquid chromatography at the Special Chemistry Laboratory of Children's Hospital Los Angeles. Urine dopamine was measured by high-performance liquid chromatography at Quest Diagnostics (San Juan Capistrano, California). Blood specimens for serum melatonin measurements were obtained and processed under dim light after 11:00 p.m. to keep the subjects' eyes from exposure to bright light, which can inhibit melatonin synthesis.

Statistical Analyses

Serial serum blood melatonin levels measured during the overnight stay were summarized as area under the curve (AUC) of melatonin vs time, calculated by the trapezoidal formula, and maximum concentration. Additional outcome measures included levels of Phe, Trp, Tyr, and other amino acids in blood and urine levels of dopamine and 6-sulfatoxymelatonin and the ratios of Phe, Trp, and Tyr to the sum of all LNAAs. Before analysis, study variables were log-transformed as needed to normalize distributions. Comparisons among the 3 phases were done with repeated-measures ANOVA, with post hoc contrasts between the washout and LNAA + placebo (LNAA) phases and between the LNAA and LNAA with Trp/Tyr supplementation (LNAA + TT) phases. Associations between blood Phe and urine 6-sulfatoxymelatonin levels were examined with Pearson correlation analysis. Statistical tests were 2-sided at a significance level of $P < .05$. Analyses were performed using SAS/STAT version 9 (SAS Institute, Cary, North Carolina).

Results

Two subjects failed to complete the LNAA + TT phase owing to discomfort, including dizziness and nausea, attributed to the Trp/Tyr tablets. For these subjects, Trp/Tyr supplementation was reduced to administer 65 mg/kg/day of Trp and 150 mg/kg/day of Tyr for the remainder of the LNAA + TT phase. One subject failed to complete the LNAA + TT phase because of poor compliance, and 1 subject accidentally took the study tablets just before a blood draw in the LNAA + TT phase, thereby invalidating plasma amino acid analysis. These data were removed from the statistical analysis. All subjects (n = 10) completed the washout and LNAA phases (LNAA and placebo supplement), 6 subjects completed the study following the protocol, and 2 subjects completed the study with a reduced amount of Trp/Tyr supplementation in the LNAA + TT phase.

Plasma Amino Acids, Serum Melatonin, and Urine 6-Sulfatoxymelatonin and Dopamine

In the 6 subjects who completed the study, levels of plasma amino acids, including Phe, Trp, and Tyr, serum melatonin, urine 6-sulfatoxymelatonin, and urine dopamine, were compared among the 3 study phases (washout, LNAA, and LNAA + TT). Phe levels were not statistically different among the 3 phases. Trp levels and Trp:LNAA were not different between the washout phase and the LNAA phase ($P = .3628$ and .1657, respectively), but were significantly higher in the LNAA + TT phase compared with the LNAA phase ($P = .0003$ and .0001). Tyr levels were higher in the LNAA